ITEM 3. INTRODUCTORY STATEMENT AND GENERAL INVESTIGATIONAL PLAN

INTRODUCTION

This investigational new drug application (IND) is being submitted in anticipation of initiating clinical trials of **ALVAC-CEA Vaccine** (NSC# 659643). ALVAC-CEA is a recombinant canarypox virus that contains the entire human carcinoembryonic antigen gene inserted into its genome. By presenting the weakly immunogenic CEA in the context of canarypox virus, our goal is to heighten the immune response to CEA in patients with CEA-expressing tumors and those populations at risk for the development of such tumors. Thus, ALVAC-CEA may prove useful both as a therapeutic and as a preventive vaccine.

NAME:	**ALVAC-CEA (or vCP248)**
CODE NAME:	**NSC# 659643**
MANUFACTURER:	**Pasteur-Merieux Serums et Vaccins (Marcy, France) / Virogenetics(Troy, NY)**
HOW SUPPLIED:	**3-cc clear glass vial which contains 0.2 mL of vaccine solution and has a labeled strength of $1 \times 10^{7.4}$ pfu/0.2 mL (equivalent to 2.5×10^{7} pfu/0.2 mL)**
STORAGE:	**Vials should be kept at -70' C or below.**
STABILITY:	**Stability studies are ongoing. Thus far, the product appears to be stable for at least 2 years when kept at -70' C.**
ROUTE OF ADMINISTRATION:	**Intramuscular**
INDICATION:	**CEA-expressing cancer**

RATIONALE

Background

Carcinoembryonic antigen (CEA) is a well-characterized tumor-associated antigen that is expressed by colonic adenocarcinomas and mucinous ovarian adenocarcinomas. CEA is also found in carcinomas of epithelial origin such as those of the breast, lung, pancreas and stomach. Low level expression of CEA has been observed in some normal colonic epithelial cells, especially those in close proximity to established tumor, while strong expression has been observed in fetal gut tissues.

CEA is a highly glycosylated monomeric glycoprotein with a molecular weight of

180,000. The CEA molecule consists of three tandemly-repeated 178-amino acid domains (Domains I, II and III). Each domain contains two disulfide bridges and is similar in structure to immunoglobulin constant loop (C2) domains. Approximately seventy percent amino acid sequence homology exists between these domains, with the four cysteine residues located at identical positions. The CEA molecule also contains an 108-amino acid N-terminus domain that is structurally related to the immunoglobulin variable domain. Sixty percent carbohydrate by weight, this molecule displays extensive carbohydrate-dependent heterogeneity. The *in vivo* function of CEA is unknown. *In vitro*, CEA can function as a homotypic and as a heterotypic adhesion molecule. Possible *in vivo* functions of CEA include (i)a role during normal embryonic colon development, (ii)a facilitator of tumor cell metastasis through adhesion processes and, (iii)through its ability to bind certain bacterial strains found in the gut, a regulatory role in bacterial colonization, as well as shedding of pathogenic bacteria.(1)

The human CEA gene belongs to the immunoglobulin supergene family and, as such, contains an anti-polar arrangement of beta strands (a typical feature of the immunoglobulin fold) as well as three disulfide bridge loop domains (see Figure 1). The CEA gene family (a subgroup of the immunoglobulin supergene family) contains CEA, non-specific cross-reacting antigens (NCA), biliary glycoproteins and pregnancy specific B_1 glycoprotein, amongst others. Corresponding domains of CEA, NCA and biliary glycoprotein exhibit 70-90% sequence similarity at the DNA level, whereas homology with pregnancy specific B_1 glycoprotein is much lower.(1)

The safety profile of the ALVAC viral vector, together with its demonstrated ability to induce both humoral and cell-mediated immune responses towards extrinsic antigens, makes it an ideal immunization vehicle for CEA. ALVAC is derived from the canarypox virus, a virus that belongs to the poxviridae family, avipoxvirus genus. Productive replication of avipoxvirus is naturally restricted to avian species and this safety feature has been preserved in ALVAC and recombinants derived from ALVAC.(2-4) The parental strain of canarypox virus (Rentschler strain) was isolated in Germany in 1970, and obtained by Institut Merieux in 1973. The virus was attenuated by 200 serial passages on primary chick embryo fibroblasts. It was registered as a vaccine for canaries in France in 1975 under the name KANAPOX (ND). No other modifications were performed on the parent organism to produce the ALVAC vector, a plaque-cloned isolate of the attenuated licensed canary vaccine. The greatly reduced virulence of the ALVAC vector has been demonstrated in three-week-old mice and newborn mice.(2) In addition, a lack of pathogenicity has been demonstrated in immunodeficient mice.(2)

Despite its highly attenuated characteristics that includes natural attenuation by host restriction, ALVAC has been shown to be an efficacious immunization vehicle. Recombinant ALVAC vectors expressing immunogens from various mammalian pathogens have been shown to induce humoral and cellular-mediated immunity in non-avian species and, where tested, to provide protection against subsequent pathogen challenge.(5-9) Even though production of virus progeny does not occur in mammalian cells, the levels of foreign gene expression attained appear to be sufficient for induction

of an immune response.

ALVAC-CEA (vCP248) is a recombinant canarypox virus that was generated by homologous recombination between a donor plasmid containing the entire human CEA gene and the ALVAC rescuing virus. It was constructed by Virogenetics Corporation in collaboration with NCI and is manufactured by Pasteur-Merieux Serum et Vaccins.

Murine Studies

The ability of ALVAC-CEA to raise a CEA-specific immune response and to prevent tumor growth *in vivo* was evaluated in a murine model developed in the Laboratory of Tumor Immunology and Biology, NCI. Briefly, a retroviral construct containing complementary DNA encoding the human CEA gene was transduced into the murine colonic adenocarcinoma cell line MC-38 B/6. Using a panel of anti-CEA monoclonal antibodies, the CEA-transduced cells were shown to express human CEA on their cell surface at a level comparable to that of human colon carcinomas. The transduced tumor cells grow rapidly in immune-competent C57 BL/6 mice, leading to the death of the host.

In the first study, two different ALVAC-CEA doses were tested for their ability to raise an immune response to CEA and to prevent tumor growth. Mice were immunized intramuscularly every two weeks for a total of three injections with either 6.25×10^{6} pfu or 2.5×10^{7} pfu of ALVAC-CEA. Two weeks following the last immunization, CEA-transduced MC-38 cells were administered to the mice subcutaneously. Both doses of ALVAC-CEA were capable of eliciting anti-CEA titers, and these titers increased upon boosting. The higher ALVAC-CEA dose tested resulted in a significantly greater titer. Interestingly, when the mice were challenged with CEA-bearing tumor cells, the differences in ALVAC-CEA dose became less apparent. Using either ALVAC-CEA dose, the majority of animals failed to develop tumors, while mice administered buffer alone developed rapidly growing tumors that eventually led to the demise of the animals. Mice that remained tumor-free for greater than 130 days were re-challenged with a three-fold greater dose of CEA-transduced tumor cells. All animals rejected the tumor a second time. One hundred and eight days following this second tumor challenge, the mice were sacrificed and submitted for extensive pathology. No neoplastic cells were noted at the implantation site or any other tissue. Moreover, there were no indications of toxicity.

A second study was conducted to assess the ability of ALVAC-CEA to boost mice previously immunized with recombinant vaccinia-CEA (rV-CEA). This may be significant in the context of a phase I clinical trial where most, if not all, patients accrued with colon cancer have already received a smallpox vaccination. Mice were initially immunized with 1×10^{7} pfu of rV-CEA by tail scratch and then boosted with 6.25×10^{6} pfu of ALVAC-CEA 0, 1 or 2 times intramuscularly; two weeks apart. Two weeks after the final immunization, mice were challenged with the CEA-positive tumor cells. According to results of an *in vitro* proliferation assay, immunized animals had a strong lymphoproliferative response to purified CEA after one vaccination with rV-CEA.

More importantly, this same response was increased still further with each succeeding boost of ALVAC-CEA. This same trend held true when measuring tumor growth and rejection. The more boosts with ALVAC-CEA, the more animals remained tumor-free and, of the mice bearing tumor, there was a greater reduction in tumor growth. Animals remaining tumor-free for greater than 150 days were sacrificed and submitted for extensive pathology. No neoplastic cells of any type were noted at the implantation site or any other tissue from any mouse and, again, there were no indications of toxicity.

In summary, immunization of mice with ALVAC-CEA can generate both humoral and cellular immune responses that are CEA-specific and dose-related. Furthermore, these responses coincide with a significant antitumor effect *in vivo.* Immunization with ALVAC-CEA provided protection against challenge with CEA-positive tumor cells. In addition, in mice primed with rV-CEA, immune responses specific for CEA can be boosted with ALVAC-CEA. It appears that prior vaccinia exposure will not prevent ALVAC-CEA from boosting a CEA-specific immune response.

Previous Human Experience

There has been no previous human experience with the ALVAC-CEA vaccine. ALVAC-CEA has not been investigated or marketed in other countries nor has it been withdrawn from investigation or marketing in any country for any reason related to safety or effectiveness.

There has been human experience with a related vaccine under our BB-IND 5041, "Human Carcinoembryonic Antigen (CEA) Vaccinia Vectored Recombinant Vaccine" or rV-CEA(180 kDa). Three clinical trials of this agent have been conducted, NCI protocols T93-0045, T92-0081 and B93-0010. Two trials were conducted in patients with adenocarcinomas of the gastrointestinal tract, breast or lung and the other trial was a pilot study in patients with low tumor burden adenocarcinoma of the colon. Recombinant vaccinia-CEA(180 kDa) vaccine was administered by scarification in three monthly injections (protocols T93-0045 and T92-0081) or as two vaccinations given eight weeks apart (B93-0010). Doses ranged from 2×10^5 pfu to 1×10^7 pfu. No serious adverse reactions were observed. Toxicity was limited to inflammation at the inoculation site and flu-like symptoms. No clinical responses were noted. However, a CEA-specific cellular immune response appears to have been generated in several patients, as indicated by *in vitro* proliferation and cytotoxicity assays.(10, 11 and Item 9)

There are several ALVAC-based vaccines currently in human trial. These include vaccines for rabies (ALVAC-RG), measles (ALVAC-MV), Japanese encephalitis virus (ALVAC-JEV) and HIV (ALVAC-HIV-1). These vaccines have been administered subcutaneously and intramuscularly and thus far no significant adverse reactivity has been found. In particular, ALVAC-RG was shown to be capable of inducing neutralizing antibody titers to levels that were previously shown to be protective in animals.(12)